

Comparative Shell Microstructure of North American *Corbicula* (Bivalvia: Sphaeriacea)

by

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Abstract. Comparative microstructural analyses of the shells of the North American "purple" and "white" forms of *Corbicula* reveal no significant differences. Shells of both forms are composed of an outer crossed-lamellar and an inner complex crossed-lamellar microstructure. Adductor myostracum in *Corbicula* is reported for the first time. The wide variation in internal shell coloration is not reflected in shell microstructure. Internal growth bands, of possible daily origin, have been found within the crossed-lamellar region of the valves.

INTRODUCTION

THE PROLIFIC NATURE of the exotic bivalve *Corbicula* sp. in North America, and its incidental fouling characteristics, has stimulated considerable efforts to understand the biology of this organism. Most previous research has assumed that a single species of *Corbicula* (*C. fluminea*) resides in North America. Recently, however, HILLIS & PATTON (1982) presented evidence that two species of *Corbicula* are found sympatrically in at least some Texas river systems. These authors based their suggestion on electrophoretic data (allelic differences at 6 of 26 genetic loci) and on morphometric data including internal shell color, shell shape, and number of shell annuli. Although the electrophoretic data are difficult to discount (and indeed are supported by additional fixed loci discovered by McLEOD [1984]), HILLIS & PATTON (1982) concede that "differences in number of annuli may reflect differences in microhabitat preferences for the two forms." In the Brazos River, Texas (locations of Hillis and Patton's *Corbicula* populations), microhabitat distinctions may include differences in water flow and siltation rates. HILLIS & PATTON (1982) typically found a form of *Corbicula* with predominantly white internal shell coloration ("white" forms) in less energetic areas with higher siltation rates, and forms with predominantly purple internal shell coloration ("purple" forms) in rocky areas with faster moving waters. PREZANT & CHALERMWAT (1984), by modifying the clam's environment in the laboratory, have found that "white" forms with purple highlights may be induced toward totally white internal shell coloration. It was also found by the latter authors (1983) that clams maintained

at warm temperatures under specific high organic conditions, as well as unhealthy or moribund animals, produced an internal crossed acicular microstructural pattern. This type of microstructure is significantly different from the typical complex crossed-lamellar pattern found in non-stressed or healthy clams of the same population. It was suggested that this modified shell type reflected stressful conditions, with animals devoting their energies toward life sustaining functions and away from normal shell production.

The taxonomic uncertainty that presently exists in North American corbiculid systematics, in conjunction with possible ecophenotypic shell modifications produced in the laboratory, prompted this comparative study of shell microstructure of the two forms of North American *Corbicula*. Scanning electron microscopic studies revealed no significant microstructural differences between shells of the two color forms but has revealed an adductor myostracum and some interesting observations on corbiculid shell formation. In addition, very subtle differences (not statistically significant in our populations with our methodology) in total organic content were noted in the two forms.

MATERIALS AND METHODS

Shells of both the "purple" and "white" forms of North American *Corbicula* were obtained from collections made in December 1981 from the San Gabriel River, Williamson County, Texas, U.S.A., by Dr. D. Hillis. Live "white" forms were collected from Tallahala Creek, Perry County, Mississippi, U.S.A. in July 1983. Soft tissues were removed from the Tallahala Creek specimens and all shells

were dehydrated in absolute ethanol for five days, followed by critical point drying in a Denton DCP-1 Critical Point Drier using liquid carbon dioxide as a transfer agent. Some specimens were free fractured prior to critical point drying. Specimens were mounted on aluminum stubs using silver paint, coated with a thin layer of gold in a Polaron SEM Coating Unit E5100, and examined on an AMR 1000A scanning electron microscope at accelerating voltages of 30 kv. At least four specimens from each group were examined.

Organic content of shell of each form (*i.e.*, Texas "white" and "purple") was determined by combustion of crushed valves in groups of four (run in triplicate) or individually (seven valves of each) for 2 h at 550°C. PALMER (1983) determined that 2 h provided optimum time for combustion of organics in shell material without significant conversion of CaCO₃ to CaO. Valves were weighed to the nearest 0.05 mg and significance tested by Student's *t*-test (two-tailed) at 2% significance level.

All figures, except Figure 1, are scanning electron micrographs.

RESULTS

Internal Coloration

The two forms, according to HILLIS & PATTON (1982), differ in several morphometric features including overall proportions (ratio of length, height, thickness) and number of shell annuli. These differences, however, are often difficult to discern (COUNTS, 1983). Of particular interest is the difference in internal shell pigmentation. According to HILLIS & PATTON (1982) the more elongated clams (greater length/height ratio) with greater number of annuli tend to possess an internal shell with a deep purple pigmentation. We have found a great deal of variation in intensity of color as well as extent of coverage. The interior of some valves is entirely purple while others have a lighter coloration over some or all the valve interior (Figure 1). In particular, the hinge teeth retain a lighter pigmentation. The full range of pigmentation in the "purple" forms runs the gamut from complete coverage of light or dark pigmentation; deeply colored only dorsal to the pallial line; deeply colored only ventral to the pallial line; deep purple highlights only beneath the umbone; or strong pigmentation only posterior to the posterior adductor scar.

Forms with stouter (lower length/height ratio) shells and fewer annuli show a lighter ("white") internal shell coloration usually with only a light purple tinge (Figure 1). Frequently, however, deeper purple coloration is found ventral to the pallial line. Specimens of "white" forms collected from Tallahala Creek, Mississippi, usually show deeper purple highlights than "white" forms collected in Texas. Larger Tallahala Creek specimens often exhibited alternating concentric bands of white and purple on the shell interior.

Specimens collected dead (*i.e.*, empty valves) are char-

acterized by a dull chalky internal shell coloration as opposed to the lustrous finish in living, healthy animals. Animals that die in the lab often have a lustrous, white internal shell coloration. Differences in these various shell forms are described elsewhere (PREZANT & CHALERMWAT, 1983, 1984).

Shell Microstructure

There were no significant differences in shell microstructure between "purple" and "white" forms from San Gabriel River, Texas, and the "white" forms from Tallahala Creek, Mississippi. All specimens possess a bilayered aragonitic shell composed of an internal complex crossed-lamellar and an outer crossed-lamellar microstructure. An evident pallial line indicates the transition zone between these two microstructures.

At high magnifications the interior surface of both shell forms ventral to the pallial line appears finely granular (Figure 2). This reflects the internal surface features of laths composing the crossed-lamellar region. In fracture section the bidirectional nature of the laths in this region is quite clear (Figure 3). Laths of this region in both color forms approximate 0.16 μm in thickness. A regular series of fine growth bands is particularly evident in radial fractures through the crossed-lamellar region near the valve edge (Figure 4) in "purple" specimens collected in December in the San Gabriel River, Texas. These bands average 9.4 μm in width with about 106 bands per mm near the periphery of a 25-mm long clam.

The inner shell layer of both forms is conical complex crossed-lamellar in microstructure (Figures 5, 6) (terminology from CARTER, 1980). Laths of this structure in both forms average 0.2 μm thick. The surficial, internal junction between complex crossed-lamellar and crossed-lamellar regions occurs in the form of a gradual transition zone over the pallial line (Figure 7). On the internal surface this zone occurs as a progression of emerging complex crossed-lamellar polygonal lath tips over smooth pallial myostracal surface (Figures 7 to 9). Lath tips range in width from 2.5 to 3.8 μm in both forms. The irregularity of direction of these tips reflects the tridirectionality of internal lamels. In fracture sections the division between the complex crossed-lamellar layer and the crossed-lamellar layer is demarcated by a zone of small prisms that often leads directly to the complex crossed-lamellar pattern externally but may also grade into a blocky zone that then leads to the complex crossed-lamellar region (Figure 10).

The surficial tips of the complex crossed-lamellar laths often decrease in size close to the umbones in "white" forms (Figure 11). Umbonal area laths are also less regular in their polygonal tip shapes. Their smoother, finer surfaces may be a result of erosion.

Infrequently, the complex crossed-lamellar surface in "purple" forms near the pallial line forms a different surficial pattern for this type of microstructure. In these cases



Figure 1

Variation in internal shell color (reflected in black, white, and shades of gray) of North American *Corbicula*. The two columns on left represent the "white" forms and those in the right columns the "purple" forms. All specimens collected from San Gabriel River, Texas, by D. Hillis.

the laths protrude farther from the plane of the shell, are less angular near their tips, and are much smaller in width (Figure 12). These laths average less than $0.5 \mu\text{m}$ in width and are extremely crowded. An apparent organic deposit is often found covering small portions of this type of surface microstructure in "purple" forms (Figure 13). This organic film forms a smooth, contoured layer filling in and "flowing" over each irregularity of this surface, and may reflect an active growth zone.

Adductor Scars

Internal surfaces of both the anterior and posterior adductor scars of the white and purple forms show concen-

tric lines (Figure 14), which appear "lighter" than the surrounding area when viewed with the scanning electron microscope. In all samples, a transition zone was observed along the peripheral region of the adductor scars nearer the umbone. The relatively smooth internal surfaces of the muscle scars merge with the irregular emerging lenses of the first order lamels of the complex crossed-lamella (Figure 15). This marks the region where the adductor scars are being grown over by complex crossed-lamellae. This is analogous to the event occurring at the pallial line. The opposing peripheral half of the adductor scars, on the other hand, did not show distinct boundaries with the outer crossed-lamellar layer.

Radial fractures through adductor scars of both white

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Explanation of Figures 2 to 10

Figure 2. Internal surface view of crossed-lamellar microstructure ventral to the pallial line near valve edge. San Gabriel white form. Horizontal field width = $14 \mu\text{m}$.

Figure 3. Fracture section through crossed-lamellar zone. Purple form. Horizontal field width = $13 \mu\text{m}$.

Figure 4. Radial fracture through crossed-lamellar region near valve edge showing periodic growth bands. Direction of growth is to the left. Purple form. P, periostracum. Horizontal field width = $940 \mu\text{m}$.

Figure 5. Radial fracture showing cone complex crossed-lamellae. Very top of micrograph shows internal surface of this region. Merger into crossed-lamellae is revealed near bottom of micrograph. Purple form. Horizontal field width = $67 \mu\text{m}$.

Figure 6. Radial fracture of cone complex crossed-lamellar zone. Purple form. Horizontal field width = $14 \mu\text{m}$.

Figure 7. Surficial view of the junction between complex crossed-lamellar (K) and crossed-lamellar (L) regions at the pallial line (B). Tallahala white form. Horizontal field width = $74 \mu\text{m}$.

Figure 8. Lath surface tips of complex crossed-lamellar microstructure. Shell edge toward top of micrograph. Tallahala white form. Horizontal field width = $32 \mu\text{m}$.

Figure 9. Complex crossed-lamellar lath tips. Shell edge toward top of micrograph. Purple form. Horizontal field width = $16 \mu\text{m}$.

Figure 10. Fracture section showing merger between complex crossed-lamellar (K) and crossed-lamellar (L) regions. Note blocky merger laths. Purple form. Horizontal field width = $27 \mu\text{m}$.

Explanation of Figures 11 to 19

Figure 11. Modified complex crossed-lamellar surface near umbone. San Gabriel white form. Horizontal field width = $16 \mu\text{m}$.

Figure 12. Complex crossed-lamellar surface just dorsal to the pallial line. Purple form. Horizontal field width = $15 \mu\text{m}$.

Figure 13. Organic deposit (O) covering surface of complex crossed-lamellar region just dorsal to the pallial line. Purple form. Horizontal field width = $8 \mu\text{m}$.

Figure 14. Internal surface of posterior adductor scar. Tallahala white. Horizontal field width = 3.4 mm .

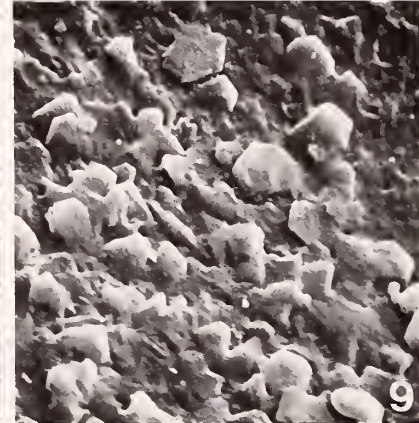
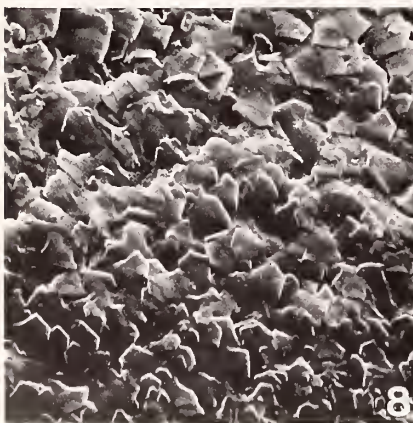
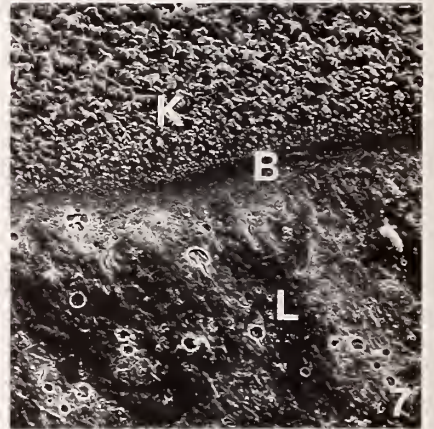
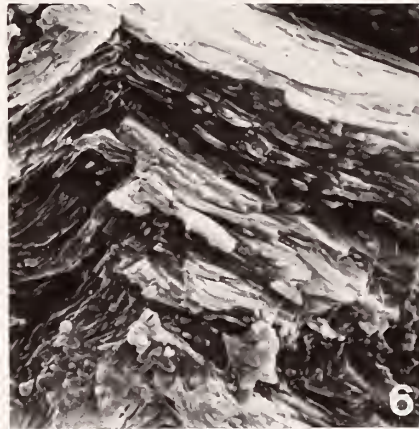
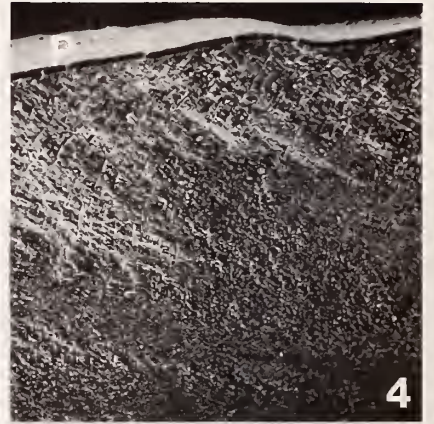
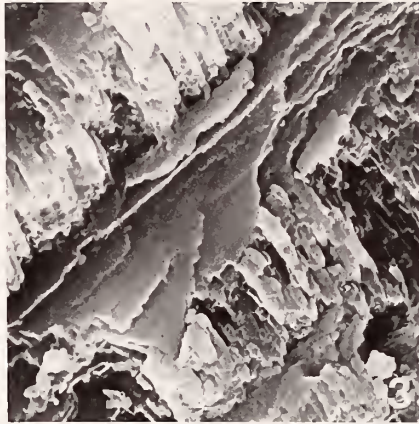
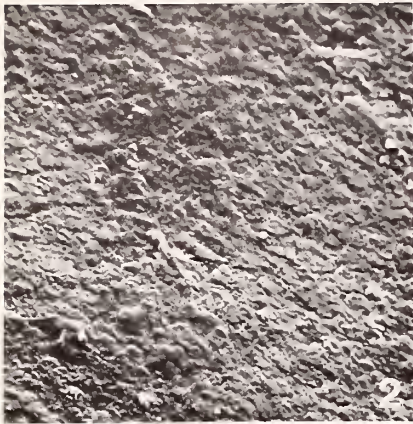
Figure 15. Internal surface of transition zone between complex crossed-lamella (K) and myostracum (A) of posterior adductor scar. Purple form. Horizontal field width = $80 \mu\text{m}$.

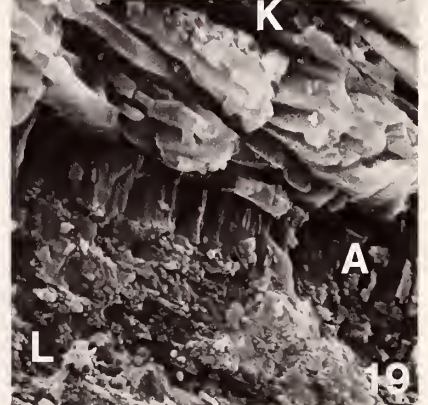
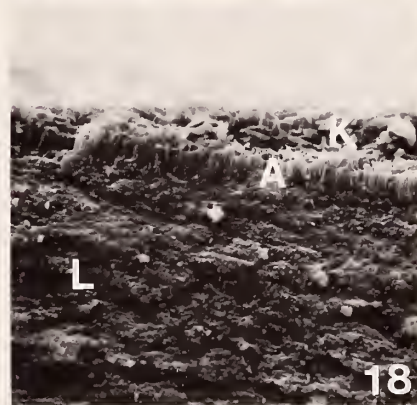
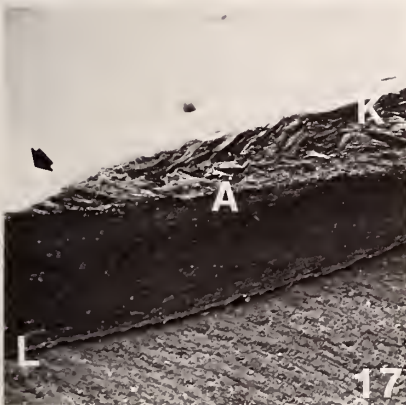
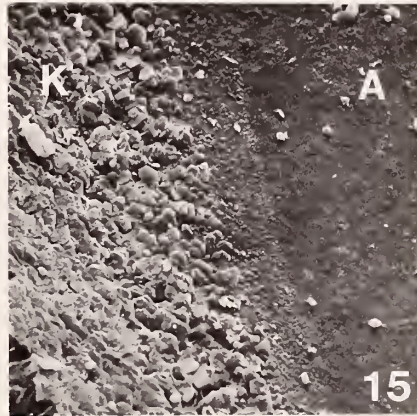
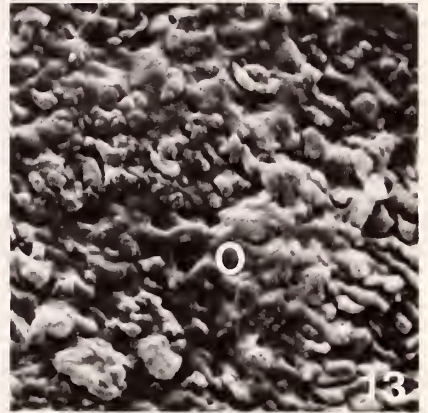
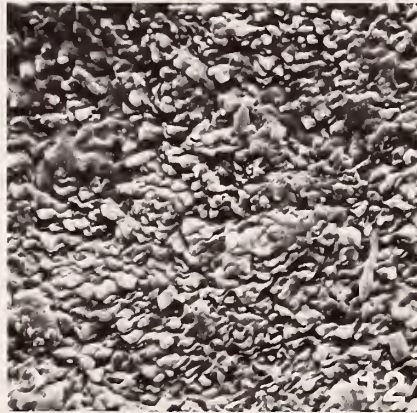
Figure 16. Radial fracture through anterior adductor scar. Myostracum (A), crossed-lamella (L). Purple form. Horizontal field width = $16 \mu\text{m}$.

Figure 17. Radial fracture through portion of anterior adductor scar (arrow) and complex crossed-lamella (K), submerged myostracum (A), crossed-lamella (L). Dark line (at L) represents internal fracture between lamels of the crossed-lamellar layer oriented in opposing directions. Purple form. Horizontal field width = $415 \mu\text{m}$.

Figure 18. Radial fracture dorsal to posterior adductor scar. Part of complex crossed-lamella (K), myostracum (A), crossed-lamella (L). Tallahala white form. Horizontal field width = $33 \mu\text{m}$.

Figure 19. Radial fracture dorsal to anterior adductor scar. Complex crossed-lamella (K), myostracum (A), crossed-lamella (L). Purple form. Horizontal field width = $13 \mu\text{m}$.





and purple forms revealed a thin internal surface prismatic layer, about 1.7 μm high (Figure 16), composing the adductor scars. Submerged myostracal prisms are embedded between the internal complex crossed-lamella and the outer crossed-lamella extending from the transition zones toward the umbones (Figures 17 to 19).

Shell Organics

Results of organic combustions of individual valves varied between 1.06 to 6.62% and between 2.32 and 2.70% for bulk determinations. Purple forms averaged slightly more overall organic shell content than white forms, but both methods (*i.e.*, bulk and individual valve determinations) yield averages that are not significantly different at the 2.0% level using a Student's *t*-test. For bulk determinations, the white valves averaged ($\bar{x} \pm 1$ SD) 2.45% \pm 0.05 shell organics and the purple valves 2.50% \pm 0.19. Individual valve determinations showed an average for the white form of 2.49% \pm 1.07 and 3.30% \pm 1.87 for purple forms.

DISCUSSION

While some morphometric and biochemical differences between "purple" and "white" forms of North American *Corbicula* may be significant taxonomic features to indicate separation of the two forms into species (HILLIS & PATTON, 1982; MCLEOD, 1984) (although COUNTS [1983] showed statistically significant overlaps between purple and white forms based on shell morphometry), shell microstructure cannot be added to the list. MACKIE (1978) suggests that "habitat type and temperature do not affect the type of crystals formed in sphaeriacean shells and that crystal type is under genetic control." The microstructural shell differences between the two forms examined here are subtle and likely reflect individual variations. These variations may reflect growth stages, erosive patterns, or modified microenvironments. The present research has revealed, however, some important features of *Corbicula* shell microstructure (*i.e.*, growth bands, adductor myostracum, *etc.*) and stimulates some interesting questions concerning biomineralization.

CARTER (1979) suggested that crossed-lamellar shell structures might yield important "signatures" for addressing taxonomic and phylogenetic problems among extant and fossil bivalve mollusks. PREZANT & CHALERMWAT (1983) have shown, however, that some complex crossed-lamellar microstructures are plastic and may be "shaped" by basic physiological conditions. There is great import in this microstructural shell flexibility and great care must be taken when using these shell features in approaching taxonomic problems in mollusks.

Differences in organic content between the two forms determined with the methods used here are not statistically significant at the 2% level using a Student's *t*-test. Although the reported differences in shell organics be-

tween white and purple forms from Texas show no statistical significance, this does not necessarily preclude the potential biological importance reflected in the slightly higher average organic content of purple valves. "White" forms showed slightly less overall shell organic content than "purple" forms; this might reflect microhabitat differences. "Purple" forms, found in faster moving waters (HILLIS & PATTON, 1982), may produce a thicker periostracum, or the organic difference may reflect an organic nature of the purple pigmentation. Further studies are needed to elucidate the significance of any organic differences.

Of interest to biomineralogists are the gradual transitions noted between the two major microstructural forms found in *Corbicula* shell. The growth of complex crossed-lamellar laths apparently proceeds with small "nuclei" being deposited as incipient laths. These are gradually added to and form the final polygonally tipped, elongate laths. Directed growth toward a central focus outside the plane of the shell accounts for the conical nature of this structure.

To our knowledge there have been no reports of internal shell growth lines in *Corbicula* prior to this study. LUTZ & RHOADS (1977) and GORDON & CARRIKER (1978) have clearly shown the relationship between growth lines in bivalves and subdaily shifts in pH, and shell dissolution. The high regularity of growth bands in *Corbicula* may indicate a process of active calcareous shell deposition alternating with growth stoppage. There is no evidence of irregular internal shell banding patterns that would indicate an active seasonal period of shell production disrupted by irregular periods of shell dissolution in the Texas population examined. We are presently investigating the possibility that the growth increments are of a daily nature.

Adductor myostracum was not observed in *Corbicula* "fluminea" from Japan, *C. occidentis* Deshayes from India, *Corbicula* sp. from Lake Nyanza (TAYLOR *et al.*, 1973) nor *C. fluminea* examined by MACKIE (1978). However, this shell layer was consistently present in samples we examined. Furthermore, there are no significant differences in microstructure of adductor scars between "white" and "purple" forms of *Corbicula*.

The arrangement of growth lines on the adductor scars suggests that the anterior adductor is migrating anteroventrally, while the posterior adductor is migrating posteroventrally as they grow. The mantle on the leading edge of the adductor is responsible for the formation of the myostracum whereas the mantle on the trailing edge produces the covering complex crossed-lamella. Adductor scar growth lines suggest localized areas rich in organic material.

The phenomenal spread of *Corbicula* in North America (McMAHON, 1982) is sound testimony to the general adaptiveness of this bivalve. The lack of distinguishing microstructural features between the two North American

forms of *Corbicula* may be indicative of plesiomorphic characters that are well established, successful, and unaltered within the family. This concurs with the basic microstructural shell trends found at the superfamily level by KENNEDY *et al.* (1969). The similarities, on the other hand, may reflect a taxonomic basis for retaining a single species for the two forms. The total evolutionary and taxonomic status of North American Corbiculidae is yet to be completely discerned.

ACKNOWLEDGMENTS

Many of the corbiculid valves used in this study were graciously supplied by Dr. David M. Hillis. The manuscript has been improved by the constructive comments of Dr. R. McMahon, Mr. K. Chalermwat, and three anonymous reviewers. Many thanks also to Ms. E. Henderson for typing the manuscript and Ms. S. DuBois for dark-room assistance.

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